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Full Length Research Paper

The effects of sewage sludge used as fertilizer on agronomic and chemical features of bird's foot trefoil *(Lotus corniculatus* L.) and soil pollution

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This study was conducted to determine the effects of sewage sludge using fertilizer at different doses (3, 6, and 9 ton da^{-1}) on birds' foot trefoil yields and chemical features. Residual of some heavy metal (Cd, Pb, Cu and Co) and macro element (K, Ca, Na, S, C and Mg,) concentrations were measured in soils and plants. According to the results of the study, sewage sludge, when applied to the soil with increasing amounts, raised the mineral matter content of plant, which affected the growth of plant favorably. Consequently, it affected the high herbage yield and increased the yield in parallellism with increasing doses of sewage sludge. Heavy metal contents were found under the critical levels in soil and plant. So, sewage sludge application as fertilizer used in appropriate doses did not pollute the soil that much, but leads to an increase in plant products.

Key words: Sewage sludge, macro elements, heavy metals, birds foot trefoil, and fertilization.

INTRODUCTION

Land application of sewage sludge has been a worldwide agricultural practice for many years. It effectively disposes the waste product while recycling valuable nutrients into the soil-plant ecosystem; however, too often, the dispersal has created environmental problems that force the government agencies to restrict the amount and type of sewage sludge to be applied. The ratio of sewage sludge usage is 36% in agricultural lands in the EU Countries (Isgenc and Kinay, 2005).

As sewage sludge contains about 50 - 70% of organic matter and a significant amount of nutritional elements in dry matter, its utilization as a source of soil organic matter and organic fertilizer has increased considerably in recent years. It has been reported earlier that the nutritional value of sewage sludge is comparable to manure and organic compost (Tabatabai and Frankerberger, 1979; Sommers, 1997) and that it contains almost all elements necessary for plant growth (Linden et al., 1983).

In many studies, it has also been shown that through application of appropriate rates of sewage sludge and compost, plant growth and physical properties of soil improve substantially, and their re-usable nutritional values increase (Reed et al., 1991). They have also maintained that by the addition of sewage sludge, a slight increase occurs in Cu content of soil and Zn content of plant, suggesting that application of sewage sludge on agricultural lands should be carried out without surpassing the toxic limits set by the relevant organizations. Furthermore, it has been reported that in soils cultivated for long, trace elements such as Zn and Cu may become deficient (Martens and Westermann, 1991) and that sewage sludge treatment can be useful in eliminating the deficiency of these metals (Logan and Chaney, 1983). On the other hand, since sewage sludge contains high concentrations of potentially toxic elements such as Zn, Ni, Cd, and Cu, problems may arise when sludge is applied to an agricultural soil (Sanders et al., 1986; Omran and Waly, 1988; Sanchez-Monedore et al., 2004; Madyiwa et al., 2002), and heavy metal accumulation in the plant tissues may also occur. The fertility benefit must be balanced against the potential hazards

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Table 1. Some features of sewage sludge used as fertilizer.

Parameter	Test result	Unit	Method
Coloring	Dark brown		DIN EN ISO 7887
Odor	musty		DIN 38 403-B1/2
Consistency	solid		Sensonical
Total solids	39.5	%	DIN 38 414-S2
Volatile solids	50.8	%	DIN 38 414-S3
Total Phosphorus	5710	mg/kg TS	DIN EN ISO 11885
Total Nitrogen	1600	mg/kg TS	DIN 19684-4

(HSE: Hamburger stadtentwaesserung testing laboratory, Hamburg, 2004).

of metal contamination through application of sludge to agriculturally productive land.

The presence of heavy metals and the risk related to resistant organic contaminants can be taken under control through restriction of the sludge amount applied on soil. Also, the presence of restrictive values on the heavy metals content of sludge is the first step in planning and designing the use of sludge in agriculture.

In our study, in order to investigate the potential use of final sewage sludge collected from urban waste treatment plant within the Diyarbakır Province, the contents of K, Ca, Mg, Na, S and C, as well as heavy metals (Cd, Pb, Cu and Co) and residual amounts of elements found in the soil after usage, were analyzed.

MATERIALS AND METHODS

Agronomic procedure

In this study, birds foot trefoil (*Lotus corniculatus* L.) was used as plant material. The experiment was designed as a pot experiment with three replications. There were three pots per treatment and in total, twenty four pots were used. As fertilizer, sewage sludge compost was provided from a wastewater refinement facility of Municipality of Diyarbakır. Some features of the sewage sludge compost are given in Table 1.

Twenty four pots were divided into eight groups: Control group was not given any fertilizer, N group was given 2 kg^{-da} N fertilizer, S1 group was given 3 ton^{-da} sewage sludge, S2 group was given 6 ton^{-da} sewage sludge, S3 group was given 9 ton^{-da} sewage sludge, N+S1 group was given 2 kg^{-da} N fertilizer and 3 ton^{-da} sewage sludge, N+S2 group was given 2 kg^{-da} N fertilizer and 6 ton^{-da} sewage sludge, N+S3 group was given 2 kg^{-da} N fertilizer and 9 ton^{-da} sewage sludge. All pots were irrigated regularly. The irrigation intervals were 7 - 10 days. The cuts were made at the 10% stage of flowering, in which all the plants were harvested in a short distance from the soil surface. After harvesting, green herbage yields were weighted. Then the herbage were dried in greenhouse conditions and weighted. Lastly, dry herbage was ground with a mill for chemical analysis. The soil used in pot experiments has a loamy structure, unsalted (E.C. 1.1), middle alkali (pH 7.9), low lime (1.64 %), low organic matter (1.44%), middle level P (14.9 mg/L) and high K (350 mg/L) (Anonymous, 1997: Basbag et al., 2004).

Data were subjected to One-Way ANOVA. Means were separated by using the Student Newman Keuls multiple comparison tests at 95% probability level.

Chemical procedure

Reagents

HNO_3, HCl, and H_2O_2 solutions were all of AnalaR grade (analytical grade substances). The standard solutions of various elements and birds foot trefoil sample solutions were prepared by using bidistilled water. The accuracy of the method was studied by examining the Standard Reference Materials Tomato Leaves-1573a. None of them contains all of the elements. For quantitative analysis, the element Standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg/L of the given element supplied by Sigma and Aldrich and stored in polyethylene bottles. All the plastic and glassware were cleaned by soaking them in dilute HNO_3 (1 + 9), and were rinsed with distilled water and dried before use (Yildiz et al., 1998).

Instruments

The inductively coupled plasma-optical emission spectrometer (ICP-OES) used was a Perkin Elmer Optima 2100 DV with axial viewing configuration. Details of the operating conditions are summarized in Table 2. A Model ATI UNICAM 929 (UNICAM, England) flame atomic absorption spectrometry (FAAS), equipped with ATI UNICAM hollow cathode lamps, was used for the sample analysis. The measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set according to the recommendations of the manufacturer. The optimum instrumental conditions are given in Table 2. From time to time, FAAS measurements were also performed to check the reliability of the data. Microwave MWS-2 closed vessel microwave system (max. temperature: 260°C) was used for microwave digestion.

Collection and Storage of birds foot trefoil samples

Thus, birds foot trefoil samples were analyzed for their concentrations of Co, Cu, Cd, Pb, Mg, Na, Ca, K, C and S.

Digestion procedures

Two types of digestion procedures were applied to the birds foot trefoil samples; wet digestion (with HNO_3-HCl) and microwave digestion (with HNO_3- H_2O_2 in microwave oven).The precision was calculated on three replicates for all digestion procedures. Optimum digestion conditions are thus explained.

One gram of sample was placed into a high form of porcelain crucible for dry digestion. The furnace temperature was slowly

Table 2. Instrumental details and operating conditions for ICP-OES Optima 2100 DV and ICP-OES wavelength (nm) of Cu, Mg, Co, Pb and Cd.

View	Axial view
Optical system	Echelle
Power/W	1450
Plasma gas flow/L min-1	15
Auxiliary gas flow/L min-1	0.2
Detector	Liquid state detector
Sample flow rate/mLmin-1	1.5
Nebulizer Nebulizing Chamber	Cyclonic
Nebulizer	Concentric glass (Meinhard) type A
Elements	Wavelength, λ (nm)
Cu	327.393
Mg	285.213
Co	228.616
Pb	220.353
Cd	228.802

Table 3. Instrumental operating parameters for FAAS (in Emission).

Parameter	Na	K	Ca
Wavelenght (nm)	589.0	766.5	422.7
Slit width(nm)	0.2	0.2	0.2
Acetylene flow rate (L/min)	0.5	0.5	0.5
Air flow rate (L/min)	4.0	4.0	4.0
Background correction	Off	Off	Off

increased from room temperature to 370℃ for 1 h. The sample was ashed for about 2 h until a grey or black ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 and, when necessary, the mixture was heated slowly to dissolve the residue. The solution was transferred to a 10 ml volumetric flask and made up to volume. The reference materials were exposed to the same procedure.

Preliminary studies have shown that mixtures of HNO_3/ H_2O_2 are better than either HNO_3 or HCl or binary mixtures of HNO_3 and HCl or H_2SO_4 in terms of complete dissolution in a short time for wet digestion. Various parameters were optimized for mineralization of birds foot trefoil sample including temperature, dissolution time and composition of HNO_3/ H_2O_2 (2/1) acid mixture.

The composition of HNO_3/ H_2O_2 (2/1) acid mixture varied during the mineralization of 1 g of a birds foot trefoil sample by heating at 110℃ for 55 min. The best results were obtained with the acid mixture ratio of 2:1 HNO_3/ H_2O_2 acid mixture for wet digestion. Increase of H_2O_2 amount from 2:1 to 1:1 did not influence the mineralization of birds foot trefoil sample for wet digestion (Yildiz et al., 1998). The Standard reference materials were treated in the same way.

In this study, we have standardized a dissolution procedure by using HNO_3 and H_2O_2 for the determination of Co, Cu, Cd, Pb, and Mg by ICP-OES. Na, Ca and K were analyzed in emission by FAAS. S and C were analyzed with ELTRA CS 500 Carbon Sulphure Determinator. Approximately 0.2 - 0.4 g of the sample was digested with 3 ml of HNO_3 and 2 ml H_2O_2 in microwave digestion system. The temperature program was as follows: 8 min at 130℃ (power 80 W), 5 min 155℃ (power 80 W) and 12 min at the maximum temperature of 170℃ (80 W). The resulting solutions were cooled and diluted to 50 ml with dezionized water. The entire procedure was checked for accuracy. Each analytical run, including also, Standard reference materials with known concentrations of Cu, Co, Cd, Mg, S, C, Na, Ca, K and Pb was carried out by using the same procedures. The clear solutions were analyzed by ICP-OES and FAAS. Working conditions and instrumental parameters of the two techniques are summarized in Tables 2 and 3, respectively.

Analysis of standard reference materials

Standard Reference Materials (Tomato Leaves-1573a) were brought into solution following the improved dissolution method and were analyzed. As it can be seen, microwave-assisted digestion results are found to be in good agreement with the certified values as seen in Table 4. In all experiments, the precision of the measurements was specified with a relative standard deviation within 0.2 - 2.3% and the relative standard deviation for three digestion replicates of each sample was ranged 1 - 10%.

Calibration

For calibration, standard solutions were prepared from the stock standard solutions of 1000 µg g^{-1} by dilution and with %1 HNO_3. The ranges of the calibration curves (7 points) were selected to match the expected concentrations (0 - 5 µg g^{-1}) for all the elements of the sample investigated by ICP-OES. Linearity was checked in the range of 0 - 40 µg g^{-1}. Detection limits were calculated as the concentrations of an element that gave a signal equal to three times the standard deviation of a series of ten successive measurements of the blank solution at the element peak (Korkmaz et al., 2007).

RESULT AND DISCUSSION

The effects of different doses of sewage sludge applied to soil on green and dry herbage weight of birds foot trefoil are given in Figures 1 - 2. As shown in Figures 1 – 2, there was a significant effect of sewage sludge treatments on green and dry herbage weight of birds foot

Table 4. Accuracy assessment through the analysis of the human hair BCR certified reference material, No.397 and tomato leaves, 1573a (µg g^{-1}, dry wt.).

Tomato leaves, 1573a			**Tomato leaves, 1573a**		
Element	**Certified**	**Found[c]**	**Element**	**Certified**	**Found[c]**
Cu	4.70 ± 0.14[b]	4.68± 0.18 [d]	Na	136 ± 4	137 ± 0.53
Mn	246 ± 8[a]	247 ± 2	K*	2.70 ± 0.05	2.68 ± 0.01
Cr	1.99± 0.66[a]	1.92 ± 0.13	Ca*	5.05 ± 0.09	5.02 ± 0.04
Fe	368 ± 7[a]	372 ± 0.9	Mg*. [nc]	1.2	1.183 ± 0.01
Co	0.57 ± 0.02[a]	0.59 ± 0.04	P	0.216 ± 0.004	0.218 ± 0.01
Cd	1.52 ± 0.04	1.51 ± 0.02	N	3.03 ± 0.15	3.06 ± 0.24
Zn	30.9 ± 0.7	31.6 ± 0.8	S* [nc]	0.96	0.93
Hg	0.034 ±0.004	0.032 ± 0.001			

* : in %; [a] : İnformative value; [b] : İndicative value; [c] : Average of three determinations; d : Mean ± SD; nc : not certified.

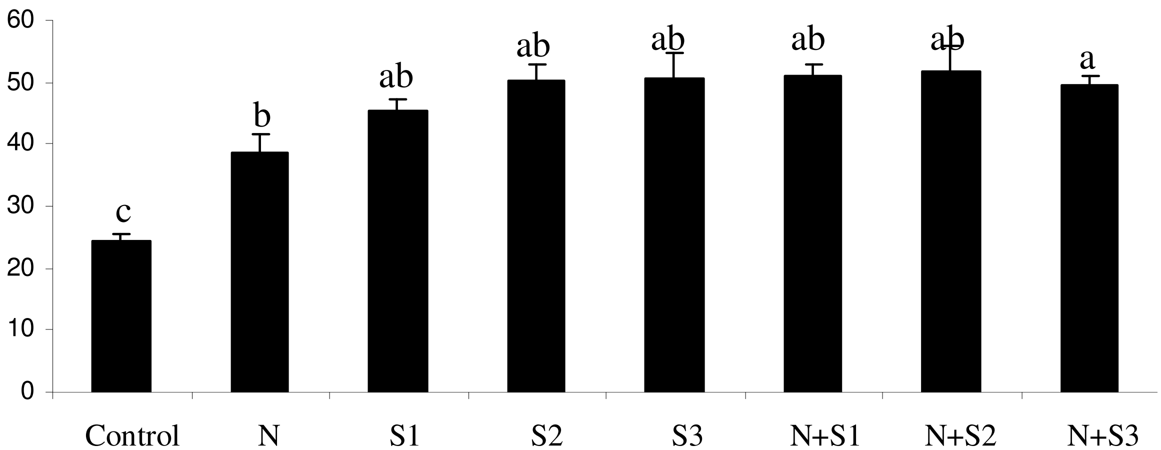

Figure 1. Effect of different doses of sewage sludge on green herbage weight (g/pot) of birds foot trefoil. Mean separation letters indicate significant differences at $P < 0.05$ between green herbage yields of birds foot trefoil.

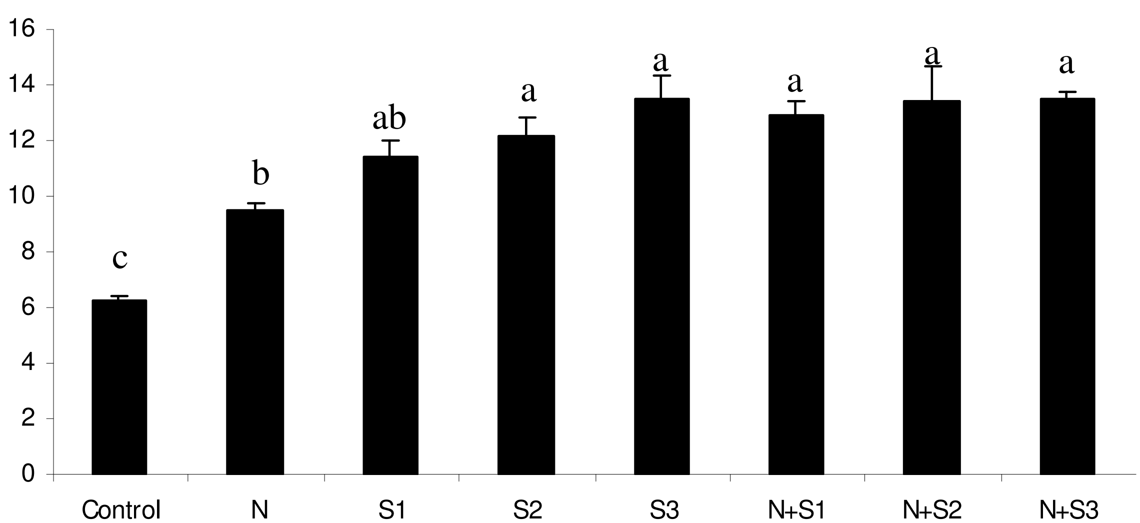

Figure 2. Effect of different doses of sewage sludge on dry weight (g/pot) of birds foot trefoil. Mean separation letters indicate significant differences at P < 0.05 between dry herbage yields of birds foot trefoil.

Table 5. Macro elements and heavy metals contents in plants.

	K (mg kg^{-1})	Ca (mg kg^{-1})	Na (mg kg^{-1})	Mg (mg kg^{-1})	C (%)	S (%)	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Cu (mg kg^{-1})	Co (mg kg^{-1})
Control	160.4 ± 2.5	208.7 ± 3.1	115.7 ± 1.1	80.5 ± 0.4	39.4 ± 0.1	0.25 ± 0.01	ND	ND	0.20 ± 0.01	0.24 ± 0.02
N	293.6 ± 1.7	292.5 ± 1.8	117.7 ± 0.7	85.4 ± 0.9	39.4 ± 0.4	0.28 ± 0.02	0.05 ± 0.0001	0.03 ± 0.0001	0.26 ±0 .03	0.33 ± 0.04
S1	353.3 ± 2.9	344.1 ± 4.8	140.3 ± 0.8	104.9 ± 1.0	39.4 ± 0.2	0.30 ± 0.02	0.08 ± 0.0002	0.27 ± 0.0050	0.39 ± 0.04	0.42 ± 0.02
S2	390.0 ± 3.1	369.6 ± 4.2	156.3 ± 1.2	116.7 ± 1.7	39.9 ± 0.5	0.43 ± 0.01	0.10 ± 0.0005	0.42 ± 0.0040	0.47 ± 0.07	0.56 ± 0.05
S3	419.3 ± 4.0	395.2 ± 5.0	185.7 ± 1.1	145.6 ± 2.2	40.86 ± 0.7	0.44 ± 0.03	0.16 ± 0.0009	0.69 ± 0.0082	0.51 ± 0.07	0.63 ± 0.03
N+S1	361.3 ± 1.2	259.2 ± 2.6	133 ± 0.8	96.8 ± 1.2	40.2 ± 0.1	0.31 ± 0.02	0.04 ± 0.0002	0.18 ± 0.0010	0.34 ± 0.02	0.22 ± 0.01
N+S2	392.6 ± 3.8	350.4 ± 3.5	144.7 ± 1.5	116.3 ± 1.8	40.5 ± 0.6	0.33 ± 0.03	0.09 ± 0.0003	0.20 ± 0.0015	0.41 ± 0.03	0.36 ± 0.01
N+S3	400.6 ± 5.2	387.7 ± 4.7	177.0 ± 1.7	139.7 ± 2.4	41.1 ± 0.3	0.34 ± 0.03	0.14 ± 0.0008	0.40 ± 0.0036	0.48 ± 0.09	0.47 ± 0.06

Table 6. The residues of macro elements and of heavy metals in soil.

	K (mg kg^{-1})	Ca (mg kg^{-1})	Na (mg kg^{-1})	Mg (mg kg^{-1})	C (%)	S (%)	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Cu (mg kg^{-1})	Co (mg kg^{-1})
Control	329.11 ± 8.0	418.16 ± 7.0	256.00 ± 6.0	41.08 ± 0.9	5.67 ± 0.2	0.076 ± 0.001	ND	ND	0.116 ± 0.02	0.101 ± 0.03
N	410.28 ± 5.0	528.82 ± 10.0	301.74 ± 8.0	80.16 ± 2.1	5.82 ± 0.4.0	0.089 ± 0.003	0.08 ± 0.01	0.11 ± 0.01	0.191 ± 0.03	0.163 ± 0.05
S1	528.66 ± 7.0	718.21 ± 15.0	400.00 ± 11.0	149.16 ± 2.8	15.16 ± 0.8	0.196 ± 0.002	0.40 ± 0.02	1.786 ± 0.11	0.342± 0.05	0.256 ± 0.07
S2	688.47 ± 9.0	926.18 ± 12.0	716.00 ± 14.0	168.18 ± 3.4	21.28 ± 0.9	0.216 ± 0.004	0.90 ± 0.13	2.987 ± 0.24	0.486 ± 0.15	0.289 ± 0.09
S3	789.16 ± 12.0	1124.12 ± 13	989.00 ± 18.0	200.1 ± 4.0	23.12 ± 1.1	0.326 ± 0.008	1.30 ± 0.19	3.716 ± 052	0.612 ± 0.11	0.312 ± 0.22

trefoil ($F = 11.796$, df = 7, 23, $P < 0.0001$; $F = 15.291$, df=7, 23, $P < 0.0001$).

The highest green herbage (51.91 g/pot) and dry herbage (13.50 g/pot) weights were obtained from N+S3 application, followed by N+S2, N+S1, S3, S2 and S1, respectively (Figure 1). The lowest green herbage (24.39 g/pot) and dry herbage (6.27 g/pot) weights were obtained from control group that did not receive any fertilizer. It was determined that increasing doses of sewage sludge and addition of N, increased herbage yield.

The data in regards to some elements and heavy metals determined within the plant structure at the end of sewage sludge application are given in Table 5. Based on the analysis of the tables, it was observed that the amount of elements transferred into the plant increased along with the rise in waste sludge, and also through nitrogen application, the amount of these elements was determined to be higher with respect to the controls.

Moreover, through an additional nitrogen application with sewage sludge, a decrease was observed in the amount of elements incorporated into the plant parts. The decrease of uptake of particularly heavy metals is of utmost importance in reducing metal content within the plant.

The measurements performed at the end of applications with regard to element residues in soil are seen in Table 6. The effect of sewage sludge applications on soil pollution is already known. Therefore, the accumulation of heavy metals in soil above the permitted limits is a major reason for banning such applications. When Table 5 was analyzed, it was observed that the content of heavy metals determined in the soil is within the limits. The permitted limits of Turkey according to pH 5 - 6, content of some heavy metals limits are Pb 50 mg kg^{-1}, Cd 1 mg kg^{-1}, Cr 100 mg kg^{-1}, Cu 50 mg kg^{-1}, Ni 30 mg kg^{-1}, Zn 150 mg kg^{-1}, Hg 1 mg kg^{-1} and Pb 300 mg kg^{-1}, Cd 3 mg kg^{-1}, Cr 100 mg kg^{-1}, Cu 140 mg kg^{-1}, Ni 75 mg kg^{-1}, Zn 300 mg kg^{-1}, Hg 1.5 mg kg^{-1} as pH > 6 (Anonymous, 2001).

Increasing amount of sewage sludge applied to soil causes plant growth and increase the content of plant nutrient in soil and plant. This is caused by chemical content and amount of plant nutrients it provided to the soil by sewage sludge. Some researchers have reported that Sewage sludge and waste materials that have a positive effect on plant growth, increased avaliable nutrient content of soil and could be a source of plant nutrients and organic matter for soil (Vieira, 2001., Ma. del Mar Delgado et al., 2002; Lopez-Mosquera et al., 2002; Asik and Katkat, 2004). These results are in compliance with our study.

Results

As a result, sewage sludge compost used at suitable doses is a valuable fertilizer for high yields and nutrient value. Also, because of suitable ratio of residues of heavy metals in soil after application, this sewage sludge fertilization may be favorable. However, dose of applied sewage and residual levels of toxic elements in soil is also important for environmental pollution. So, we have identified that the residual levels of heavy metals in soil are not line-out for allowable limit. Besides, we observed that the application of N into soil increased the amount of macro elements, while the amount of heavy metals in plant structure decreased considerably.

Potassium

The plants take up the highest potassium from S3 fertilizer (419.3 mg kg^{-1}), the value of potassium slightly decreased with additional nitrogen (400.6 mg kg^{-1}).

Calcium

The plants take up the highest calcium from S3 fertilizer (395.2 mg kg^{-1}), the value of calcium slightly decreased with additional nitrogen (387.7 mg kg^{-1}).

Sodium

The plants take up the highest sodium from S3 fertilizer (185.7 mg kg^{-1}), the value of sodium slightly decreased with additional nitrogen (177.0 mg kg^{-1}).

Magnesium

The plants take up the highest magnesium from S3 fertilizer (145.6 mg kg^{-1}), the value of magnesium slightly decreased with additional nitrogen (139.7 mg kg^{-1}).

Sulfur

The plants take up the highest sulfur from S3 fertilizer (0.44%), the value of sulfur slightly decreased with additional nitrogen (0.34%).

Carbon

The plants take up the highest Carbon from N+S3 fertilizer (41.1%), the value of carbon slightly decreased without nitrogen fertilizer (40.86%).

Cadmium

The plants take up the highest cadmium from S3 fertilizer (0.16 mg kg^{-1}), the value of cadmium slightly decreased with additional nitrogen (0.14 mg kg^{-1}).

Lead

The plants take up the highest lead from S3 fertilizer (0.69 mg kg^{-1}), the value of lead slightly decreased with additional nitrogen (0.40 mg kg^{-1}).

Copper

The plants take up the highest copper from S3 fertilizer (0.51 mg kg^{-1}), the value of copper slightly decreased with additional nitrogen (0.48 mg kg^{-1}).

Cobalt

The plants take up the highest cobalt from S3 fertilizer (0.63 mg kg^{-1}), the value of cobalt slightly decreased with additional nitrogen (0.47 mg kg^{-1}).

As a summary, appropriate dose of sewage sludge used as fertilizer increase the yield of plants. However, using the appropriate dose of sewage sludge is very important in avoiding soil pollution. Particularly, residue levels of heavy metals in soil should not exceed the critical levels, and adjusting doses of fertilizer should be taken into account.

In our study, it was observed that application of up to 9 $tons^{-da}$ of sewage sludge resulted in an increased yield of plants, and critical levels of heavy metals in soil was protected. The application of sewage sludge also showed increased amounts of nutrients in soil. So, this kind of applications would be useful in terms of increased productivity in soil having poor organic matter.

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